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DEGENERATION OF PERIPHERAL NERVES [1]

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TWO PLATES

It is customary in the routine work after an autopsy to examine the peripheral nerves of a beriberic or "suspect" for fatty degeneration of the myeline sheath by the Marchi method, after which a final diagnosis is made. Therefore a person who is not aware of the possible presence of degeneration in nonberiberics and who is not in the habit of examining nerves of cases other than beriberics will often be misled in his conclusions. If the degeneration of the peripheral nerves is accompanied by œdema of the subcutaneous tissue with moistness, congestion, and petechiæ of the serous surfaces, marked dilatation and hypertrophy of the right ventricle, congestion and œdema of the lungs, congestion of the viscera, and acute duodenitis, then a diagnosis of beriberi is justified. However, it is possible that the above morbid anatomy may be present in cases which are clinically nonberiberic, with the exception of a right-sided hypertrophy. On the other hand, such an important finding as hypertrophy of the right ventricle may be and is often absent in the acute cases of beriberi. Œdema is a constant symptom in the course of beriberi, but may be so trivial in the acute pernicious form, in the rudimentary form, and in the late stages of the disease that it becomes unrecognizable at autopsy. A previous attack of typhoid, pneumonia, diphtheria, or any acute infectious disease may leave sufficient degeneration in the heart muscle to cause sudden cardiac dilatation. Acute cardiac dilatation also occurs in pernicious anæmia, chronic interstitial nephritis, and generalized arteriosclerosis. It is in these cases of sudden deaths of which a clinical history is often lacking that the morbid anatomist will be confronted with difficulties, and the mere presence of

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fatty degeneration in the myeline sheath does not justify the diagnosis of beriberi.

The object of this work is twofold: namely, to determine the presence of degeneration in the peripheral nerves of morbidities other than beriberi and to emphasize the presence of the degeneration in nonberiberics.

This paper will treat the subject from the viewpoint of morbid anatomy only, without reference to the clinical history or course of each case studied. It is unfortunate that comparatively few cases of frank beriberi came to the morgue during the period of this study, so that only six could be included in this series. No attempt will be made to compute the incidence of degeneration in terms of percentage, due to lack in number in each class of cases studied.

TABLE I.—*Sources from which material is derived.*[a]

	Cases.
Philippine General Hospital	48
St. Joseph's Hospital	16
San Lazaro Hospital	27
"Outside"	13
Total	104

TABLE II.—*Interval between death and autopsy.*

	Cases.
Under 12 hours	44
12–24 hours	46
24–36 hours	8
36–48 hours	1
Over 48 hours	5
Total	104

TABLE III.—*Age of cases studied.*

	Cases.
Under 1 year	7
1–5 years	10
5–20 years	14
20–50 years	56
Over 50 years	17
Total	104

TABLE IV.—*Classification of cases according to clinical diagnosis.*

Clinical diagnosis.	Cases.
Pulmonary tuberculosis	16
Asiatic cholera	26
Accidents (acute anæmias, fractures, etc.)	10
Cardiac dilatation	4
Beriberi	3
Acute ileocolitis	4

[a] The Philippine General Hospital supplied surgical and infectious cases. St. Joseph's Hospital supplied tuberculous cases. San Lazaro Hospital supplied contagious cases. "Outside" supplied accidents, medicolegal, and undetermined cases.

TABLE IV.—*Classification cases according to clinical diagnosis*—Continued.

Clinical diagnosis.	Cases.
Submersion	3
Bronchopneumonia	3
Lobar pneumonia	1
Surgical shock	1
Undetermined	3
Acute nephritis	1
Cystolithiasis	2
Biliary cirrhosis of the liver	1
Uræmia	1
Typhoid	1
Intestinal tuberculosis	2
Tuberculosis of the liver(?). Carcinoma(?)	1
Cerebral hemorrhage	1
Tuberculous meningitis	2
Carcinoma of the neck and viscera	1
Strangulated hernia	1
Ascariasis (cholera?)	1
Pulmonary œdema	1
Carcinoma of the liver	1
Empyæma	1
Pulmonary hemorrhage (tuberculous)	1
Purpura fulminans	1
Atrophy	1
Acute malaria	2
Puerperal infection	2
Noma (cancrum oris)	1
Electrocution	1
Acute suppurative cholecystitis	1
Hemorrhage from gastric ulcer	1
Chronic nephritis and suppurative pyelitis	1
Total	104

TECHNIC

From 2 to 4 centimeters of the vagus and posterior tibial nerves are laid on a strip of cardboard and treated as follows:

1. Harden in equal parts of Müller's fluid and formaldehyde (10 per cent) for twenty-four hours.
2. Replace by Müller's fluid and keep in it for fifteen days.
3. Wash in running water for from twelve to twenty-four hours.
4. Transfer the tissue for fifteen days to the following solution:

	cc.
Müller's fluid	2
2 per cent osmic acid solution	0.5
Distilled water	0.5

5. Wash in running water for from twelve to twenty-four hours.
6. Dehydrate in graded alcohols, changing the absolute alcohol twice.
7. Clear in oil of origanum, tease under a dissecting microscope, and mount in chloroform balsam.

It is not necessary to place the nerves from one case in separate bottles and label accordingly, because the posterior tibial is always two to three times larger than the vagus nerve.

Surplus tissues after step 3 are placed in 80 per cent alcohol for future staining if necessary.

A record of the gross conditions of all the organs, tissues, and serous surfaces examined was tabulated with the autopsy number, age, number of hours after death the autopsy was performed, nutrition, clinical diagnosis, cause of death, and the findings in the peripheral nerves. This tabulation simplified computation and classification and made apparent the coincidence of degeneration in certain organic lesions. Table V, giving the causes of death, shows the occurrence of degeneration in all the cases studied.

An arbitrary designation of the extent of degeneration was used in the tables, for instance, + + + for marked degeneration, + + for moderate, + for slight, and — for the absence. The accompanying illustrations at the end of this article show these designations clearly. The type and degree of degeneration in the vagus are identical with those of the posterior tibial.

TABLE V.—*Incidence and degree of degeneration in all the cases studied.*

Cases.	Cause of death.	+++	++	+	—
25	Enteritis (cholera)		7	16	2
14	Pulmonary tuberculosis		5	7	2
10	Accident (violence)		2	4	4
6	Cardiac dilatation (beriberi)		3	3	
4	Bronchopneumonia			1	3
3	Lobar pneumonia		1	2	
2	Ulcerative colitis (amœbic)			2	
3	Acute suppurative peritonitis			2	1
2	Acute malarial infection	1	1		
2	Acute suppurative endometritis			2	
2	Carcinomatosis		1	1	
10	Cardiac dilatation (acute)	1	2	5	2
1	Generalized tuberculosis				1
1	Sarcoma of right kidney			1	
1	Enteritis (typhoid)			1	
1	Pyonephrosis			2	
1	Cirrhosis of the liver			1	
1	Lymphosarcoma of stomach			1	
2	Intestinal obstruction			2	
1	Tuberculous peritonitis			1	
1	Marasmus (atrophy)		1		
1	Multiple chloroma	1			
1	Membranous colitis		1		
1	Acute vegetative endocarditis	1			
1	Chronic, interstitial and parenchymatous nephritis		1		
1	Hemorrhage from gastric ulcer		1		
1	Suppurative cholecystitis		1		
1	Gangrenous stomatitis			1	
1	Tuberculous meningitis		1		
2	Submersion		1		1
	Total	4	29	55	16

Table V shows that in a mixed autopsy service in Manila 88 out of 104 cases showed microscopically degeneration of the peripheral nerves by the Marchi method. Of these 88 cases the degeneration was slight in 55, moderate in 29, and marked in 4.

TABLE VI.—*Incidence of degeneration and its degree in different ages.*

Cases.	Age limits.	+++	++	+	—	Total.
7	Under 1 year		3	2	2	7
10	1 to 5 years			8	2	10
14	5 to 20 years		5	8	1	14
56	20 to 50 years	2	17	30	7	56
17	50 and over	2	4	7	4	17
	Total	4	29	55	16	104

Table VI shows that of the 88 cases of nerve degeneration more than half (49) occurred between the ages of 20 and 50 years, but no age was exempt.

TABLE VII.—*Classification of kidney conditions with the incidence and degree of degeneration.*

Cases.	Condition of kidneys.	+++	++	+	—	Total.
41	Acute parenchymatous nephritis and acute parenchymatous degeneration	2	13	20	6	41
32	Chronic interstitial nephritis	2	11	16	3	32
7	Congestion		3	3	1	7
6	Decomposed		1	3	1	5
5	Anæmia			3	1	5
1	Tuberculosis				1	1
5	Normal			3	3	6
2	Sarcoma			2		2
3	Pyonephrosis			3		3
2	Chronic parenchymatous nephritis			2		2
	Total	4	29	55	16	104

Table VII shows that of the 88 cases of nerve degeneration 69 had nephritis of some form.

TABLE VIII.—*Classification of cases to show condition of nutrition in terms of number of cases and incidence of degeneration.*

Cases.	Poorly nourished.	Fairly well nourished.	Well nourished.	Total.
Without degeneration (—)	5	4	7	16
With slight degeneration (+)	13	10	32	55
With moderate degeneration (++)	13	1	15	29
With marked degeneration (+++)	1		3	4
Total	32	15	57	104

TABLE IX.—*Cause of death and interval before autopsy in cases with good nutrition and with (+) degeneration.*

Cases.	Cause of death.	Hours after death.
3	Acute cardiac dilatation	8, 19, 8
1	Ulcerative colitis	27
1	Cardiac dilatation with stenosis (mitral)	35
2	Acute anæmia	22, 7
1	Fractures of pubic and sacral bones	24
1	Bronchopneumonia	21
1	Lobar pneumonia	19
1	Suppurative peritonitis	4
1	Pulmonary tuberculosis	7
1	Cirrhosis of the liver	5
1	Intestinal obstruction	3
13	Enteritis (cholera)	17, 19, 31, 19, 7, 8, 4, 18, 21, 19, 14, 12, 31
1	Acute anæmia from carotid	14
1	Occipitovertebral dislocation	(?)
2	Suppurative endometritis	3, 15
1	Infantile beriberi	5
32		

TABLE X.—*Cause of death and interval before autopsy in cases with fair nutrition and with (+) degeneration.*

Cases.	Cause of death.	Hours after death.
1	Acute cardiac dilatation	3
1	Suppurative peritonitis	22
1	Enteritis (typhoid)	23
1	Tuberculous pneumonia	25
1	Pyonephrosis	10.5
1	Intestinal obstruction	10
1	Tuberculous peritonitis	12
1	Cardiac dilatation with mitral stenosis	29
1	Enteritis (cholera)	9
1	Lobar pneumonia	24
10		

TABLE XI.—*Cause of death and interval before autopsy in cases with poor nutrition and with (+) degeneration.*

Cases.	Cause of death.	Hours after death.
5	Pulmonary tuberculosis	5, 16, 15.5, 9.5, 22
1	Pyonephrosis	22.5
1	Sarcoma of right kidney	23
1	Primary carcinoma of liver	7
1	Lymphosarcoma of intestine	12
1	Ulcerative colitis	13
2	Enteritis (cholera)	5, 9
1	Gangrenous stomatitis	3
13		

TABLE XII.—*Cause of death and interval before autopsy in cases with poor nutrition with marked and moderate degeneration.*

Cases.	Cause of death.	Degeneration.	Hours after death.
1	Multiple chloroma	+++	14
5	Pulmonary tuberculosis	++	22.5, 16.5, 82(?), 58, ?
1	Lobar pneumonia	++	17
1	Beriberi	++	19
1	Atrophy	++	19
1	Acute malaria	++	4
1	Suppurative cholecystitis	++	15
1	Hemorrhage from gastric ulcer	++	51
1	Carcinomatosis	++	12
1	Chronic interstitial and parenchymatous nephritis	++	6
14			

TABLE XIII.—*Cause of death and interval before autopsy in well-nourished cases with marked and moderate degeneration.*

Cases.	Cause of death.	Degeneration.	Hours after death.
8	Acute catarrhal enteritis (cholera)	++	5, 15, 9.5, 7, 19, 2, 3, 16
2	Acute anæmia from stabs	++	14, 3
1	Submersion	++	48
1	Electrocution	++	16
1	Bacillary dysentery	++	15
1	Acute malaria	+++	18
1	Cardiac dilatation with arteriosclerosis	+++	14.5
1	Tuberculous meningitis	++	2
1	Acute beriberi (adult)	++	10
1	Vegetative endocarditis with arteriosclerosis	+++	4
1	Infantile beriberi	++	15
19			

Tables VIII to XIII show that the condition of nutrition is not an important factor in determining the nerve degeneration and that a reasonable interval (two to forty-eight hours) between death and autopsy does not necessarily mean a sequential myeline sheath change as demonstrated by the Marchi method. (These bodies were kept at a temperature just above freezing.)

The effect of decomposition on nerves was studied by the application of the usual technic to pieces of tissue which have been kept wrapped in muscle at room temperature and fixed at varying intervals. Table XIV shows that degeneration either persists or disappears as decomposition advances.

TABLE XIV.—*Showing the effect of decomposition.*

Autopsy No.	Hours after death.	Degeneration.	Hours after death.	Degeneration.	Hours after death.	Degeneration.	Hours after death.	Degeneration.	Sheaths.
5103	3	+	22	+	38	+	.....	.....	Stained dark.
5118	15	+	34	+	39	+	.....	.....	Dark and granular.
5120	5	+	9	+	29	—[a]	34	—[a]	Beaded and fragmented.
5402	16	+	40	—[a]	.....	.....	.....	.....	Beaded and fragmented.

[a] This disappearance is due to a rapid solution of the reduced osmic acid in the clearing oil.

TABLE XV.—*Degeneration in six decomposed cases studied.*

Autopsy No.	Cause of death.	Degeneration.	Hours after death.
4548	Submersion	—	21
4858	Pulmonary tuberculosis	—	40
4864	Generalized tuberculous peritonitis	+	12 (?)
4881	Enteritis (cholera)	+	31
5003	Submersion	++	48
5102	Occipitovertebral dislocation	+	(?)

Table XV shows the persistence of degeneration in advanced decomposition.

The results of this work show that degeneration of the myeline sheath in the peripheral nerves in morbidities distinctly nonberiberic is not only present but frequent, and such frequency should always be borne in mind during all post-mortem investigations.

The frequency of degeneration in old age associated with generalized arteriosclerosis and chronic interstitial nephritis, in Asiatic cholera, in pulmonary tuberculosis, in conditions with poor nutrition, and even in apparently healthy individuals killed by accidents is evident, although no definite conclusions could be drawn due to a lack in number in each class of cases studied.

The following explanations have been offered by others as etiological factors in the degeneration of the myeline sheath of peripheral nerves:

1. Degeneration of peripheral nerves may occur in any disturbance of metabolism and nutrition as a result of chemical poisoning or parasitic toxins. (1)
2. Degeneration of peripheral nerves results from a deficiency of a substance or substances necessary for a normal metabolism. (2) (3) (4)
3. Degeneration in apparently healthy individuals is probably due to a one-sided diet (white rice) of the lower class of people to which the

cases studied belong. This explanation is sustained by the experiments in fowl by Vedder and Clark, from which they drew the following conclusions:

a. Degeneration is present as early as the seventh day of polished rice feeding.

b. Degeneration may be present without symptoms of neuritis even after thirty-five days of feeding.

c. Degeneration occurs before the symptoms.

d. Advanced degeneration may be present with no symptoms of neuritis.

4. Unbalanced diet may be responsible for degeneration in those who died of accidents or diseases of short duration, the degeneration being independent of and preceding the disease.

Osler(4) gives the following causes under the etiology of multiple neuritis:

(*a*). The poisons of infectious diseases, as in leprosy, diphtheria, typhoid fever, small-pox, scarlet fever, and occasionally in other forms; (*b*) the organic poisons, comprising the diffusible stimulants, such as alcohol and ether, bisulphide of carbon and naphtha, and the metallic bodies, such as lead, arsenic, and mercury; (*c*) cachectic conditions, such as occur in anæmia, cancer, tuberculosis, or marasmus from any cause; (*d*) the endemic neuritis of beri-beri; and (*e*) lastly, there are cases in which none of these factors prevail, but the disease sets in suddenly after overexertion or exposure to cold.

According to this author neuritis in tuberculosis is not common, while in typhoid fever parenchymatous changes have been met with in the peripheral nerves and appear to be not very uncommon even when there have been no symptoms of neuritis.

In bacillary dysentery peripheral neuritis, though often confined to one nerve, is not uncommon in the mild forms of the disease.(6)

SUMMARY AND CONCLUSIONS

1. In a mixed autopsy service in Manila 88 out of 104 cases showed microscopically degeneration of the peripheral nerves by the Marchi method. Of these 88 cases the degeneration was slight in 55, moderate in 29, and marked in 4.

2. Of the 88 cases of nerve degeneration more than half (49) occurred between the ages of 20 and 50 years, but no age was exempt.

3. Of the 88 cases of nerve degeneration 69 had nephritis of some form.

4. The condition of nutrition is not an important factor in determining the nerve degeneration, and a reasonable interval (two to forty-eight hours) between death and autopsy does not necessarily mean a sequential myeline sheath change, as demonstrated by the Marchi method. (These bodies were kept at a temperature just above freezing.)

With the knowledge that this study was made from a routine autopsy service in Manila and that the subjects were all Filipinos, the following factors must be taken into consideration in attempting to explain the occurrence of degeneration in 88 out of 104 cases studied.

1. In Manila beriberi is endemic, and therefore there is the possibility of many unrecognized cases of mild beriberi dying of other diseases.

2. There may be many cases who recovered from beriberi and died of other diseases.

3. The nutrition of the working class of Filipinos in general is below par, due to insufficient food, excessive nonnutritious food, or a one-sided diet.

It is necessary that the clinical history must aid the morbid anatomist in the diagnosis of beriberi. The morbid anatomy of this disease consists of subcutaneous œdema, moistness, congestion, and petechiæ of the serous surfaces, marked dilation of the right ventricle with or without hypertrophy, congestion and œdema of the lungs, congestion of the viscera, acute duodenitis, and degeneration of the myeline sheath of the peripheral nerves.

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(3) CASTELLANI and CHALMERS. Manual of Tropical Medicine. Ballière, Tindall and Cox, London. 1st ed. (1910), 998.
(4) OSLER. The Principles and Practice of Modern Medicine. D. Appleton & Company. New York and London. 8th ed. (1912), 1021.
(5) STRONG and CROWELL. *Phil. Journ. Sci., Sec. B* (1912), 7, 271.
(6) VEDDER and CLARK. Ibid. (1912), 7, 423.

ILLUSTRATIONS

PLATE I

Fig. 1. Normal nerve.
2. Slight degeneration (+) in a case of ulcerative colitis (amœbic).
3. Moderate degeneration (++) in a case of Asiatic cholera.

PLATE II

Fig. 1. Marked degeneration (+++) in a case of acute malaria.
2. Marked degeneration (+++) in a case of ulcerative endocarditis with generalized arteriosclerosis and jaundice.
3. Moderate degeneration (++) in a case of infantile beriberi.

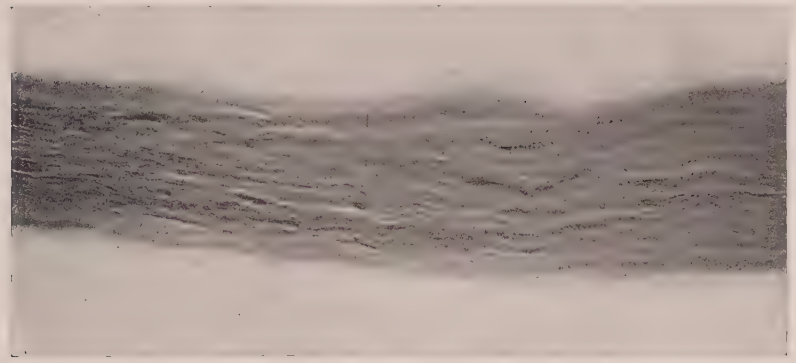

Fig. 1. Normal nerve.

Fig. 2. Slight degeneration (+) in a case of ulcerative colitis (amœbic).

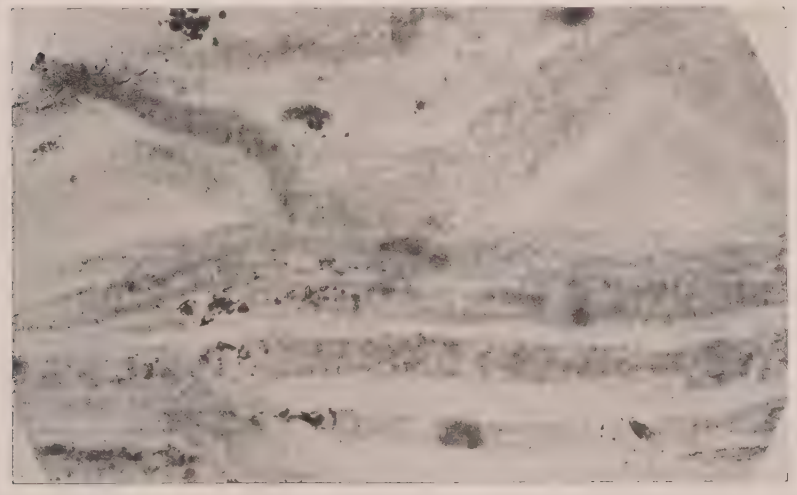

Fig. 3. Moderate degeneration (++) in a case of Asiatic cholera.

PLATE I.

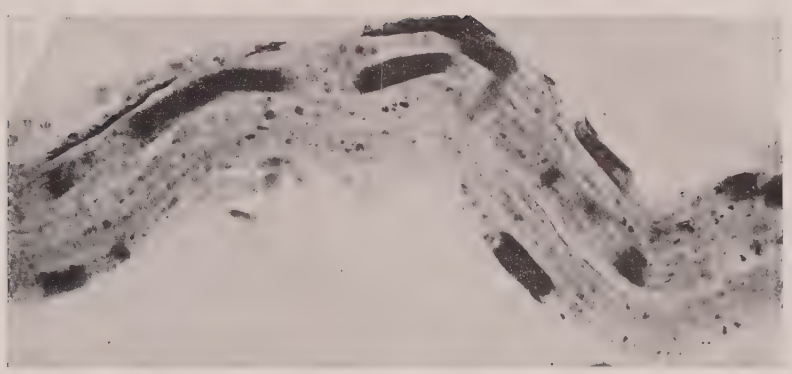

Fig. 1. Marked degeneration (+++) in a case of acute malaria.

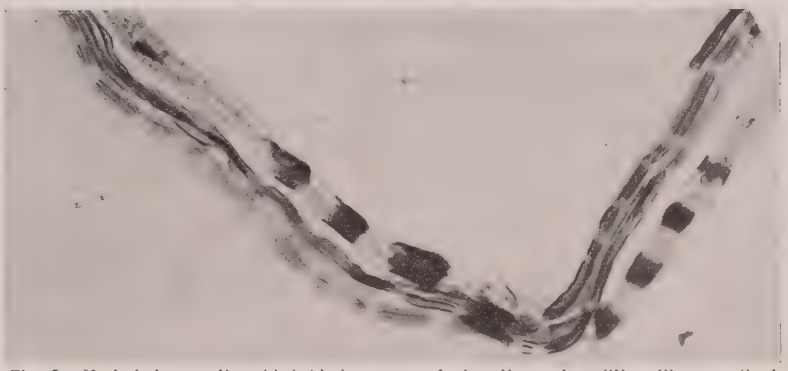

Fig. 2. Marked degeneration (+++) in a case of ulcerative endocarditis with generalized arteriosclerosis and jaundice.

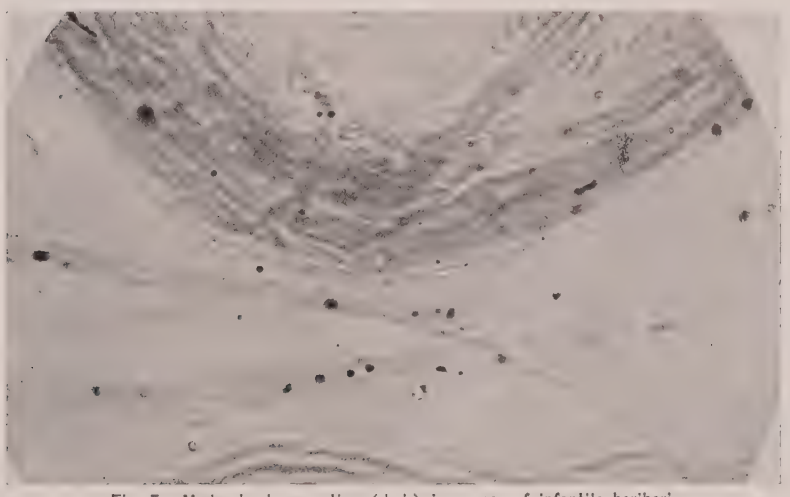

Fig. 3. Moderate degeneration (++) in a case of infantile beriberi.

PLATE II.

DISAPPEARANCE OF THE PIGMENT IN THE MELANOPHORE OF PHILIPPINE HOUSE LIZARDS [1]

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TWO COLORED PLATES

The literature that has accumulated on the subject of melanophores and chromatophores or pigment-carrying cells is abundant and is chiefly concerned with a discussion of their origin, morphology, and movements (Eycleshymer,(2) Laurens,(6) Spaeth,(10) Lieben,(7) Ballowitz,(1) Hooker,(3) and others). However, the study of the pigment granule, its elaboration, migration, and destruction, has received but comparatively little attention (Von Szily,(11) Redfield,(8) Hooker,(4) and others).

An analysis of the literature indicates that there are at least three different morphological types of melanophores, which Laurens(5) reviews at some length.

> Ballowitz * * * reiterates his many times expressed opinion that the movements of the pigment in the pigment cells are due to intracellular streaming in the chromatophores which have unchangeable cell forms. The protoplasm of the chromatophores is filled with numerous extremely fine, radially arranged, anastomosing canals in which the pigment is contained, and which are closed on the outside. No membrane can be demonstrated, but the walls of the canals are extremely thin and are formed by the protoplasm of the chromatophore. The contraction of the protoplasmic walls of the canals, alternating with its relaxation moves and drives forward the pigment granules. If the protoplasmic wall in the processes of the cells contracts in transverse waves from the periphery toward the center, then the pigment streams toward the center of the cell, and the canal protoplasm of the center of the cell disk at the same time relaxing, the pigment streams into it and rounds it off. On the other hand, if the central protoplasm contracts, and the protoplasm of the process relaxes then the melanin granules stream out into the processes.
>
> Spaeth holds to the theory, which is perhaps the most popular, that the chromatophores of fishes are fixed stellate cells within which the pigment is carried in a rather fluid cytoplasm, streams into and out of the processes during expansion and contraction.
>
> Hooker [2] upholds another and third view, that the melanophores of Amphibia * * * lie in preformed spaces and that they expand and contract within the spaces which enclose them. The acts of expansion and

[1] Received for publication January, 15th 1917.

[2] See Hooker, reference No. 3.

contraction, according to this theory, are brought about by pseudopodia, the pigment granules being carried in the cell cytoplasm and the pigment cells are therefore to be considered as amœboid.

In a more recent paper Spaeth(9) describes the melanophore as a type of smooth muscle cell and in part concludes—

> That in the case of the melanophore there is no direct evidence at hand for a loss or exchange of fluid during contraction. There is, however, a visible and reversible colloidal aggregation of melanin granules following a variety of physiological stimuli all of which elicit contractions in smooth muscle. Similarly a number of physiological stimuli that produce a relaxation in smooth muscle brings about a dispersion of the invisible colloidal particles. The aggregation of melanin granules within the melanophore must therefore be considered a visible expression of the colloidal phenomenon that occurs upon stimulation in the micro-homogeneous colloidal content of smooth muscle cell.

From the above theories and statements it is evident that there is still considerable doubt as to the finer mechanism and nature of the "contraction" and "expansion" of the melanophore, or the aggregation and dispersion of the melanin granules within the cell. Do we after all have more than one morphological type of melanophore, or does some chemical reaction take place in the cell with a subsequent disappearance or reappearance of the pigment granules that may be interpreted as an apparent contraction or expansion of the cell? In a series of experiments we have had occasion to study the pigment cells of Philippine house lizards (*Cosmybotus platurus, Peropus mutilatus, Hemidactylus frenatus,* and *Hemidactylus luzonensis*—the last very rare), and we have observed that the melanophoric changes in these animals are of a type that does not accord with the descriptions given by investigators who have worked with the other types of melanophores previously mentioned.

Our experiments have been made both upon the living lizard and on isolated pieces of skin that could be studied under the microscope. Some of our observations, along the lines recorded by other investigators, include the results of enucleation of the eyes, decerebration, severing the spinal cord, various kinds of stimulation, and the effects of physiologically active substances such as adrenalin chloride, chloretone, curare, atropine, potassium cyanide, ether, chloroform, carbon monoxide, and isotonic salt solutions.

The change of color in Philippine house lizards is effected fairly rapidly, both from a light to a dark color and vice versa, and the period of time for each change is approximately the same under normal conditions. The variation in color range is merely one of light and dark intensity and includes all the

shades between the two extremes of a creamy white to a taupe (the darkest gray) with a slight brownish tinge. Under normal conditions this lizard changes its color from one extreme to another in about thirty minutes. Upon irritation by shaking in a Petri dish, the time for changing from a dark to a light color can be greatly lessened, approximately fifteen minutes being necessary for the "contraction" of the melanophore. Redfield has observed similar results in the horned toad during states of nervous excitement and believes that this coördination of the contracting melanophore must be due to a hormone, which is probably "adrenin."

A number of experiments were performed to determine, if possible, the normal condition of the melanophore when at rest, whether it was then expanded or contracted. In one series of lizards the eyes were removed under ether anæthesia, while in others the cerebrum was removed, and in still others the spinal cord was severed. The eyeless lizards assumed a dark color irrespective of the color of the background and surroundings.

In the decerebrated lizards similar results were observed both as to their indifference to react to background stimulation, darkness, and light. However, the latter, if mechanically irritated, would always turn a light color, and the melanophores would become "contracted." Laurens says that the eyeless *Amblystoma* larvæ do not respond to difference in background, though they do respond to light stimulation; this is quite the reverse from what is found in lizards. The melanophores of the eyeless *Amblystoma* larvæ, when kept in light, are fully expanded, and when kept in the dark, the subepidermal melanophores are maximally contracted.

If it be true that there is an actual "contraction" and "expansion" of the melanophore, then the aggregated central mass of pigment granules should have greater dimensions in the "contracted" than in the "expanded" cell. In the bleached lizards, many melanophores were seen as minute pigmented points only. Other cells persist, apparently of the normal expanded type, evidently unaffected or only slightly affected in the normal bleaching reflex. However, we have also made measurements of the central masses in such persisting cells in cleared sections of skin; when compared with similar measurements of the central masses of the melanophores of the normally dark lizard, the former were usually less and never greater than the latter.

When cleared sections of dark, light, and the intermediate shades of lizard skin were examined under the oil immersion, the details of the melanophore could be easily seen. The center

of the cell was composed of a mass of pigmented granules; radiating pseudopods filled with granules extended in all directions. In the skin sections taken from lizards in which the melanophores were "contracted," it was observed that the peripheral portion of the pseudopod was composed of *farblose* granules. The melanophore of the lizard is, then, a definite fixed stellate cell composed of granules which may be either pigmented or colorless. "Farblose" and "colorless" are not used in their literal meanings; we use them as terms indicating the appearance of granules that have been depleted of their dark pigment. These bleached granules have a slight yellowish tinge.

Hooker further states,

> It may safely be concluded that, in the normal ontogenetic origin of melanin in the frog the chromatin plays no direct role. On the contrary, all the evidence obtained goes to demonstrate that melanin granules are formed in the cytoplasm from elements already present in solution in it, through some action of the nucleus.

To arrive at some definite conclusions regarding these pigmented and farblose granules, a series of experiments was carried out. It was believed that if a means could be found to destroy the pigment, an actual observation could be made under the microscopic. For these experiments small fragments of skin, 4 millimeters square, were removed from the back of a lizard; the pieces of skin were then spread and floated on the surface of an adrenalin solution. The strength of the solution used was 1: 10,000 (1 part of adrenalin chloride to 10,000 parts of Ringer's solution). As soon as the specimen was prepared, it was placed under the microscope and it was observed that the granules in the periphery of the pseudopod gradually began to fade. This fading process progressed rapidly toward the center of the cell. Some melanophores were completely depleted of their pigment, others showed the central pigmented mass remaining, and still others had scattered fragments or groups of pigmented granules separated entirely from the center. The stroma of the pigmented cell remained in situ, clearly visible, and of a pale yellow color. These are also shown in the section of cleared skin from the lizard bleached normally within white surroundings (Plate I).

In the above experiments, the skin was floated on the surface of the solution. This was found necessary, since the results were not as constant when fragments of skin were submerged. Local application of adrenalin to the skin of a dark lizard in dark surroundings results in the prompt fading of the area that is moistened by the solution (Plate II, fig. 1).

Lieben was perhaps the first investigator to record the action of adrenalin on the melanophore and states:

> Gelegentlich einer Adrenalininjektion bei einem Frosche zum Zwecke der Studiums ihrer Wirkung auf die Gefässe fiel mir auf dass sich die Pigmentzellen sehr bald nach Beginn des Versuches ballten und der ganze Frosch hell wurde.

The adrenalin that we used was diluted in Ringer's solution, 1:10,000, and 0.1 cubic centimeter was injected into the dorsal lymph sac of a normal dark lizard. Two minutes later there was a distinct blanching of the localized area overlying the injected sac, following which there was a gradual systemic fading of the color of the lizard, so that in from ten to fifteen minutes following the injection the entire skin was a creamy white (Plate II, fig. 2). Throughout the experiment the lizard was kept in dark surroundings. Control animals injected with Ringer's solution and placed in the same chamber showed only a slight fading of the skin immediately surrounding the point of injection. In numerous similar experiments the lizards always reacted promptly when newly prepared adrenalin solutions were employed.

We have tried in many ways to study other conditions that might effect changes in the melanophore. Many of them have been repetitions of those recorded by other investigators. Experiments with ether and chloroform vapor, in vivo and in vitro, were not conclusive. Carbon monoxide experiments resulted similarly, though dark lizards placed in illuminating gas were slightly bleached because of irritation attending efforts to escape. Adrenalin placed on the skin or injected into the lymp sac was still effective in anæsthetized and carbon monoxide lizards. Skin placed in calcium saline or in potassium saline solution did not change. Experiments with curare, atropine, and potassium cyanide were negative. Application of the tetanizing current to isolated pieces of skin was without effect. Exposure of the lizards to temperatures between 0° and 30° C. produced no change. Dark, dorsal skin, ground with sand and strained through cloth, was not affected by adrenalin. Isolated pieces of skin, killed by exposure to chloroform vapor, did not react to adrenalin.

Some interesting observations were obtained by the administration of chloretone. When light-colored lizards in white surroundings were injected in the dorsal lymph sac with 0.1 cubic centimeter of 1 per cent chloretone solution, the skin overlying the injected area soon became more and more pigmented (Plate II, fig. 4). Subsequently the entire lizard gradually became

darker. The process of pigmentation is much slower than the change from dark to light effected by adrenalin.

The area first darkened by the injection remained well defined in several experiments, indicating a direct effect on the melanophore cells, though positive pigmentation of the granules could not be demonstrated when pieces of light-colored skin were floated on chloretone solution under the microscope. Injection of adrenalin or application of adrenalin to the surface of the skin of chloretonized lizards gives the characteristic bleaching.

DISCUSSION

Investigators have heretofore described small pigment-carrying granules in chromatophores and melanophores. Von Szily refers to them as *Pigmentträger* and says:

> Die Umwandlung der farblosen Pigmentträger im Pigment erfolgt wahrscheinlich unter dem Einfluss von spezifischen Zellfermenten. Die Letzteren können ihre Wirkung auf das Chromatin, die Muttersubstanz des Pigmentes, erst dann ausüben wenn die Kernmembran normalerweise im Verlaufe der Mitose zeitweise verschwindet, oder wenn einzelne Chromatinpartikelchen in der Teilungsruhe unter den eben beschriebenden Umständen aus dem Kern eliminiert werden.

No other workers have referred to the colorless pigment granules, or farblose Pigmentträger. Hooker and Spaeth seem convinced that there exist in the cytoplasm elements that are capable of combining and by some influence of the nucleus are transformed into melanin granules. Hooker concludes that—

> In the cells of embryo frogs, melanin is formed from some substance (probably tyrosine or its derivatives) in solution in the cytoplasm when acted upon by the nucleus (perhaps an oxidase reaction).

What rôle is played by the nucleus in the production of the pigment in the granules of the lizard we are at present not prepared to state. However, studies are now being carried on that may throw more light on this subject. If melanin granules become pigmented by means of an oxidase, we can conceive also of the gradual destruction and disappearance of the pigment by chemical action. This process of destruction and production of pigment explains the apparent "contraction" and "expansion" of the melanophore in the lizard.

Our experiments show conclusively that normally the coördinate fading of the melanophore is due to a primary nervous reflex, through stimulation of the retina, with probably a subsequent production and liberation of a hormone, which is carried about by the blood and lymph. What this hormone is we are

unable to say, though everything points toward "adrenin," as Redfield has previously indicated. Adrenalin has a definite action on the pigment of the melanophore in both the in vitro and in vivo experiments; there results a gradual fading of the pigment from the periphery toward the center of the cells.

CONCLUSIONS

1. The melanophore of Philippine house lizards (*Cosmybotus platurus, Peropus mutilatus, Hemidactylus frenatus,* and *Hemidactylus luzonensis*) is a definitely fixed stellate cell, which neither contracts nor expands; all pseudopods remain in situ during the fading and repigmentation processes.

2. No migration of the pigmented or farblose granules was ever observed either from the center toward the periphery of the cell or vice versa.

3. The pigment in the melanophore of the Philippine house lizards is actually dispersed and disappears from the granule on the application of adrenalin both in vitro and in vivo, leaving a definite farblose granule which can be seen with high magnification. The same farblose granules are also seen in the melanophores of the lizard bleached by white surroundings.

4. It may be assumed that the disappearance of the pigment of the granules is an intracellular chemical change, which seems to occur when the melanophore is stimulated by a hormone (probably adrenin) ; the pigmented state is the normal condition, the melanophore in vivo elaborating the pigment when the reflex secretion of the hormone is discontinued.

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ILLUSTRATIONS

PLATE I

[Drawing by Julian Castro.]

A camera lucida drawing of a melanophore of a Philippine house lizard, showing both the pigmented and the colorless granules in the radiating pseudopods. No. 3 ocular, 1/12 oil immersion objective, reduced about one third.

PLATE II

Fig. 1. A house lizard, showing the bleaching of the skin five minutes after the application of adrenalin.

2. A house lizard partly bleached by the injection of 0.1 cubic centimeter of adrenalin chloride (1:10,000) into the posterior lymph sac. The lizards shown in figs. 1 and 2 were kept in the same dark cage during the experiment.

3. A normal dark lizard.

4. A normal bleached lizard ten minutes after an injection of 0.1 cubic centimeter of 1 per cent chloretone in the right posterior lymph sac; the darkened area of skin overlies the injected lymp sac.

5. A normal lizard partly bleached by being kept in white surroundings.

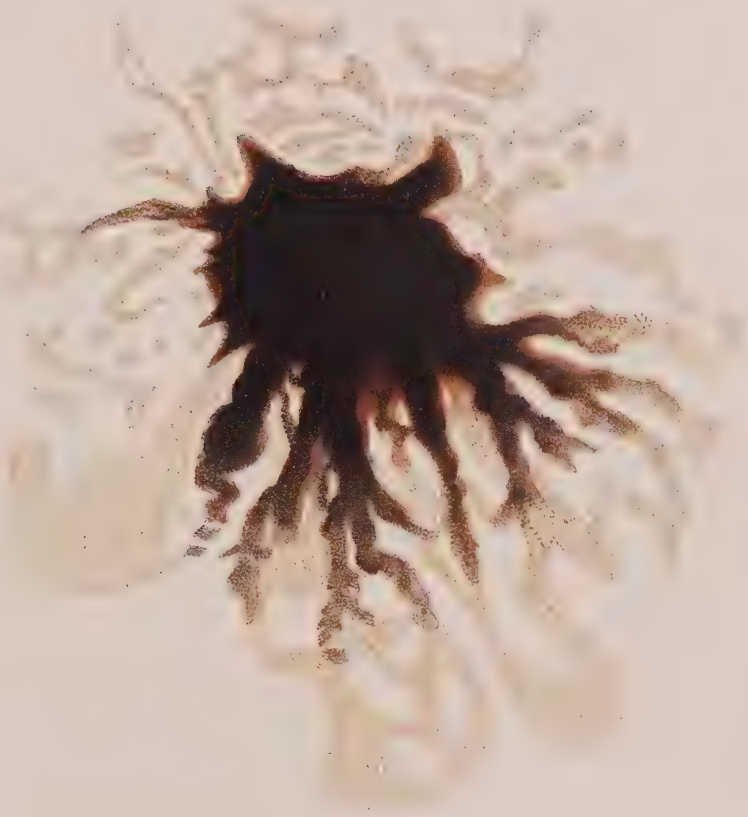

PLATE I. A MELANOPHORE OF A HOUSE LIZARD.

PLATE II. PHILIPPINE HOUSE LIZARDS.

ESSENTIAL FACTOR IN THE TREATMENT OF PREGNANT CHOLERA PATIENTS [1]

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Among the many incidents that a physician treating cholera has to contend with none, perhaps, will cause him more anxiety than the handling of the pregnant woman. The almost hopeless condition of coma in some and delirium in others, so striking at times, often causes him to give them less attention than they deserve. For over a century men have written about cholera, describing the clinical picture, and have often mentioned the pregnant cases, comparing their chances for recovery with those of the nonpregnant. However, the different workers contended with unlike conditions, and their methods of treatment varied greatly; consequently their results and deductions are so nonuniform that considerable doubt is left in one's mind as to the correct prognosis of these cases. It is evident that in order to obtain satisfactory deductions one must treat a sufficient number of these cases, of varying virulence, extending over a considerable period, and not be misled by the prognosis obtained in small epidemics.

In Manila we have had admissions of cholera to San Lazaro Hospital almost continuously from September, 1913, to February, 1917, of a spasmodic virulence varying at times from a very severe typical type to a more or less mild atypical one. The cholera here is thought by some to be endemic, and the admissions during this period numbered 1,588, all of which were Filipinos residing in Manila or its near vicinity. Of these, 379 were females between the ages of 15 and 40, and 77 of this number were pregnant, which were distributed as follows: 4 cases were pregnant for two months; 13 for three months; 4 for four months; 13 for five months; 9 for six months; 14 for seven months; 11 for eight months; and 9 for nine months.

Sixty-six of these cases received the ordinary treatment of (1) intravenous injections of a hypertonic salt solution containing soda bicarbonate and (2) the usual symptomatic treatment when

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indicated, resulting in 36 recoveries and 30 deaths, which gave a mortality of 45 per cent. This mortality is indeed high when compared with the 19 per cent obtained from the identical treatment in the 302 nonpregnant cases, that is, 245 recoveries and 57 deaths as tabulated in Table I.

TABLE I.—*Showing the admissions, recoveries, deaths, and mortality of the pregnant and the nonpregnant cases under discussion.*

	Admissions.	Recoveries.	Deaths.	Mortality.
				Per cent.
Nonpregnant	302	245	57	19
Pregnant	[a]66	36	30	45
Total	368	281	87	23

[a] Eleven cases are excluded for later consideration.

These results compel me to disagree with Kovalsky,(10) who states that the mortality in the pregnant cases is not much higher than in the nonpregnant ones. Davis(3) is of the same opinion, in that cholera does not materially increase the mortality, whereas Basil(1) states that when the pregnancy is far advanced in a cholera case it adds much to the gravity of the condition. Hirsch(8) obtained a mortality of 54 per cent; Bouchut,(2) 56 per cent; Härten,(7) in Utrecht, 58 per cent; and Freschi,(5) in Genoa, saw nearly all the pregnant cases die. Nichols and Andrews,(12) from their experiences in San Lazaro Hospital, Manila, in 1908, think that as much as pregnancy lessens the chances of the patient's recovery such cases do not necessarily prove fatal. Schütz's(16) results show a mortality of 57 per cent for the pregnant cholera patients, and he thinks that this mortality is in direct proportion to the lateness of the pregnancy. It is evident, as shown in Table I, that the chances for recovery of the pregnant cholera patient are decreased by more than twofold. These results agree with those of Liebermeister,(11) who states that pregnancy greatly increases the danger to the life of the mother.

Some authorities call attention to the fact that in cholera, in general, the mortality depends upon the interval between the attack and the time of receiving treatment. For the present 66 cases, the average interval for those who died was twenty-five hours, and for those who recovered it was fourteen hours. Most of the patients were admitted in the stage of evacuation, that is, the first stage of the disease, and the low mortality of only

17 per cent obtained for this period can be explained by the fact that the treatment administered to the cases in this period carried many over it that would have otherwise died in it. The mortality for the algid stage was 65 per cent, an important fact, which will be referred to later on, and for the third period, or the stage of reaction, it was 18 per cent.

When one considers that the system is greatly taxed during cholera and that the continuation of the pregnancy is in danger, as is found in the other acute infections, one must naturally expect abortions to occur. This was true in the present series of 66 cases, which gave a total of 37 abortions (56 per cent), which can be favorably compared with the 50 per cent reported by Bouchut.(2) For the sake of clearness the term "abortion" is used to denote any kind of delivery at any month. Liebermeister(11) and Nichols and Andrews(12) agree that the pregnant cholera patients usually abort. Hirsch,(8) in his summary of cases in 1854, gives a high percentage (78 per cent); 17 of his 25 cases aborted. Schütz(16) reports 52 abortions in 115 cases (45 per cent), calling attention to the tendency to abort more frequently as the pregnancy is advanced, which condition was present in this series, as is illustrated in Table II, but not in the arithmetical progression as stated by Schütz.

As to the stages of the disease when abortion most frequently occurs, Liebermeister(11) states that if the mother does not succumb she usually aborts during the period of reaction. The present series of cases does not support this statement, as 5 per cent of the fœtuses were delivered in the first stage of the disease, 75 per cent in the second, and 20 per cent in the third. In 1892 Klautsch(9) saw in Hamburg 10 pregnant cholera cases abort in the second stage of the disease. These results are further supported by Davis,(3) who claims that the fœtuses usually die in the second stage, the abortions setting in during the same period.

Of the 66 cases of pregnancy in the present series, 37 aborted, of which only 2 fœtuses were born alive, one of which died within a few hours after delivery, and the other was discharged from the hospital alive with the mother. All other cases were either born dead or before the age of viability; adding to this number those that died with the mother, we obtain a total of 49 fœtal deaths in 66 pregnancies (71 per cent). Kovalsky(10) states that over 80 per cent of the pregnancies in his cases came to an untimely end, and Bouchut(2) and Hirsch(8) saw equal fatal results (80 per cent) in their respective experiences.

Klautsch(9) saw 10 pregnant cases admitted to the hospital and all the fœtuses were born dead, while Schütz(16) reports that most (75 per cent) of his birth cases were stillborn. All these different observers agree that abortions are very frequent in cholera and that the fœtuses are rarely born alive. The fact that pregnant women who survive an attack of cholera later on abort, or that the children when born show any effect of the cholera with which the mother was attacked when pregnant, is contrary to the opinions of Davis(3) and Basil.(1) However, no extensive observations have been made in the present series touching on this point.

TABLE II.—*Showing fœtal and maternal mortality in pregnant cholera patients.*

	Months of pregnancy.								Total.
	2d.	3d.	4th.	5th.	6th.	7th.	8th.	9th.	
Patients who aborted:									
Deaths	2	1	1	3	2	2	5	2	18
Recoveries	0	4	1	4	3	1	0	6	19
Mortality for the mother ---- per cent	100	20	50	43	40	66	100	25	49
Average percentage for the first four months, the second four months, and the ninth month			44				60	25	49
Patients who did not abort:									
Deaths	0	3	0	1	2	3	2	1	12
Recoveries	2	4	1	2	2	4	2	0	17
Mortality for the mother ---- per cent	0	43	0	33	50	43	50	100	42
Average percentage for the first four months, the second four months, and the ninth month			30				40	100	42
Sum total for both:									
Total mortality for the mothers ---- per cent	50	33	33	40	44	50	77	33	45
Total mortality for fœtuses ---- per cent	50	66	66	80	78	60	77	88	71
Tendency to abort per cent	50	42	33	70	55	30	55	90	56
Average percentage of abortions for the first and second periods of the pregnancies			47					60	

All observers seem to agree that the weakness and the toxic condition of the mother causes a protracted and irregular delivery, which is more pronounced the longer the period of pregnancy

and the greater the severity of the case. The fœtuses are usually cyanotic and often show various degrees of maceration, thus indicating that death had taken place a considerable time previous to the delivery. As a rule, the fœtus, membranes, and placenta were delivered intact in direct proportion to the severity of the patient's condition and inversely to the period of gestation.

Therefore, if abortions occur in the majority of the cases of cholera and the fœtuses are stillborn, what effect do they have on the mortality of the mother? Galliard and Schütz(6) saw the majority of pregnant cholera cases die after abortion had taken place. Rumpf(14) entertains a similar opinion to Bouchut,(2) who states that abortion offers an unfavorable prognosis for the mother. Table II shows the aborting cases in the present series, which resulted in a mortality of 49 per cent for the mother, and in those cases in which abortion did not occur, 42 per cent of the mothers died. Hirsch's(8) results are almost equal for those who did not abort (63 per cent) and for those who did abort (65 per cent), but Schütz(16) obtained a much greater mortality for those who did abort, that is, 62 per cent in comparison with the 44 per cent mortality for those who did not abort.

From these percentages it seems that the patients who aborted thereby decreased their chances for recovery, whereas those who did not abort had a better prognosis and kept their fœtuses intact. But my clinical observations have inclined me to believe otherwise, and this belief is strengthened by a study of the figures of Bouchut,(2) who states that of his 18 cases which aborted only 9 died, while 19 of the 25 which did not abort died. I am convinced that the abortion is a life-saving measure for the mother when it occurs in time, although the high percentage of mortality as given by others seems to indicate the contrary. This act of abortion is a possible factor for recovery in the severe form of the disease and unnecessary in the mild form. Can we conclude that such cases would not have terminated fatally if they had not aborted? The fact that it required an average period of 9.6 days in order to recover in those cases that did abort in comparison with the average of 7.3 days required for recovery in the cases that did not abort does not signify that the abortion retarded the recovery, but indicates that this class of patients was of a more serious type. Although many died after abortion, it does not necessarily mean that the abortion was a cause of it but an effect of the disease and a necessary requirement for recovery and that the abortion

occurred too late to be of any avail, as the period between the admission to the hospital and the time of the abortion was an average of thirty-one hours for the fatal cases and twenty-two hours for those who recovered. The retardation of the abortion in the fatal cases naturally caused the delivery to take place at a time when the patient's condition was at the weakest, when more toxins had been absorbed from the dead fœtus, and when the energy expended in the process was sufficient completely to exhaust the mother. This was strikingly illustrated in a number of cases in which the last effort, voluntary and involuntary, made by the patient in this world was to rid herself of her dead fœtus. Further the percentage of mortality for the mother observed in favor of the cases that did not abort is in itself a proof that this class of cases was of a milder form, that the mother was not absorbing any toxins from a dead fœtus to aggravate her already serious condition, and that the energy not expended in delivering a fœtus was reserved for the recovery of herself.

The fact that the mortality for the mother is increased proportionately to the term of the pregnancy, as seen in Table II, agrees with a similar statement made by Schütz.(16) The mortality of the mothers (60 per cent) aborting fœtuses ranging from the fourth to the ninth month is greater than for the period before the fourth month (44 per cent). The larger the fœtus, the greater the tax required by the mother in its expulsion, thus lengthening the process of the delivery. The more fully developed placental circulation and the firmer attachment of the placenta during the latter months of pregnancy facilitate the absorption of toxin originating in the dead fœtus before its separation occurs, thus rendering more unfavorable the mother's prognosis.

The mortality of 50 per cent for the mother for the total of the second month of pregnancy (Table II) brings to one's mind the idea of toxæmia of pregnancy so frequently seen in early pregnancies in general, causing the cholera patient a graver prognosis, while those of the ninth month, being so near term, cause no additional strain on the mother, as convincingly seen in the resulting mortality of only 33 per cent in 10 cases. It was in this group of cases that a child was born alive to be discharged from the hospital with its mother.

From the foregoing it is evident that abortions are very frequent in cholera patients, that the mortality is very much higher in the pregnant cases, and also that the fœtuses are nearly all stillborn. The question arises, What kills the fœtus?

TABLE III.—*Showing 66 cases divided into 6 groups, based upon the presence or absence of the radial pulse on admission and the interval between the admission and the appearance of urine.*

	Radial pulse present on admission.	Radial pulse not present on admission.
Urination on admission.	(Group No. I): 23 cases; 11 abortions (48 per cent); mortality of cases, 17 per cent.	(Group No. VI): No cases in this group.
Urination was present within 24 hours after admission.	(Group No. II): 8 cases; 5 abortions (62 per cent); mortality of cases, 13 per cent.	(Group No, V): 4 cases; 3 abortions (75 per cent); mortality of cases, 50 per cent.
Urination was not present within 24 hours after admission.	(Group No. III): 21 cases; 11 abortions (52 per cent); mortality of cases, 66 per cent.	(Group No. IV): 10 cases; 7 abortions (70 per cent); mortality of cases, 80 per cent.

Group I comprised 23 cases, which had a radial pulse and urinated on admission. Of this class 17 per cent died and 48 per cent aborted.

Group II comprised 8 cases, which had a radial pulse and did not urinate on admission, but did urinate within twenty-four hours. This group showed a mortality of 13 per cent; 62 per cent aborted.

Group III comprised 21 cases, which had a radial pulse on admission, but did not urinate within twenty-four hours. The mortality for the mother was 66 per cent; 52 per cent aborted.

Group IV comprised 10 cases, which did not have a radial pulse on admission and did not urinate within twenty-four hours. The total mortality of this group was 80 per cent. Seven of the 10 cases aborted (70 per cent), and three cases did not. This group is interesting, because it portrays the worst prognosis and because the three cases that did not abort died. The two cases that aborted and recovered were pregnant for less than four months.

Group V comprised 4 cases, which had no radial pulse on admission, but urinated within twenty-four hours. The mortality for the mothers was 50 per cent, and 75 per cent aborted.

For completeness group VI was made to show the outcome of the cases which had no radial pulse but urinated on admission. As no such condition can reasonably exist, no cases were found to represent this group.

Rogers(13) has proved that by lowering the specific gravity of the blood to normal by the addition of a solution which thereby restores the blood to its normal volume and so increases the arterial pressure many patients who upon admission are pulse-

less and fail to urinate can be made to do so in a short time. Therefore the absence of urine in the early stage of cholera must be a mechanical process resulting from the fluid portion of the blood becoming decreased, and hence the remaining blood, of higher specific gravity and decreased volume, is unable to circulate through the secreting tubules of the kidneys, resulting in the cessation of urinary secretion. Then the same principle must apply to the arterial system of the uterus. If this failure of the circulation lasts sufficiently long, the fœtus must surely die, because the mother's blood fails to nourish it and to carry off the waste products, thus allowing the toxins to accumulate in it. Also the failure of the mother to eliminate properly results in the damming up of her own toxins to the detriment of the fœtus.

These factors alone are sufficient reasons for the subsequent death of the fœtus and for the high percentage of abortions as seen in group III. But then, what justifiable reasons can be given for the cases comprising group I, which cases never became pulseless and did urinate on admission, showing 48 per cent abortions in contrast with group III, which cases did not urinate on admission and gave 52 per cent abortions, a difference of only 4 per cent. Then if the cessation of urination in the early stage does not cause abortion, and as the period of the fever before abortion occurs is so short, it does not seem possible for the same factor which causes the abortions in the other fevers to be responsible. Sellards (17) has proved the presence of an acidosis in cholera, and Rogers (13) states that this acidosis is an important factor in causing postcholeraic uræmia. Davis (3) says that the placenta is affected and that the fœtal movements are unusually violent during the first stage, and my own observations agree with this statement. Schütz (16) found that cholera shows a marked tendency to affect the nonpregnant uterus, particularly in the first stage, producing a metrorrhagia in one third of the cases, but that the Graafian follicles show no signs of a hemorrhagic condition, thus dispelling the idea that this metrorrhagia may be a menstruation. This condition was not often noticed in the present series of cases, but the necropsy reports of the nonpregnant cases, many of which were not admitted to the hospital and were untreated by intravenous injections, showed that hemorrhage in the uterine cavity, hemorrhagic endometritis, and congestion of the serosa of the uterus, ovaries, and fallopian tubes were not uncommon. Slavjansky (15) has described a hemorrhagic endometritis in one third of his nonpregnant cases.

Williams(19) states that in nearly every instance the disease causes uterine contractions, resulting from the toxins in the blood. Expecting then a similar change in the pregnant uterus, a high percentage of abortions is sure to occur, and as it has been shown that they occur mostly in the second stage of the disease, the fœtus-killing factor must necessarily act very early in the first stage before the patient becomes pulseless, and so if, on admission, the fœtus is dead and any assistance is to be rendered to the mother, it must be given as early as possible, in order to cause the absorption of a minimum amount of fœtal toxins and also to cause the exhaustion of the smallest possible amount of energy in the delivery of the fœtus.

In the latter part of 1916 the cholera admissions increased, and the pregnant cases were frequently examined to ascertain if the fœtus was dead or if abortion was in progress. If either of these two conditions was present, then such assistance was given as indicated by the severity of the cases (Table IV).

TABLE IV.—*Eleven cases in which dilatation of the cervix uteri or dilatation and extraction were performed.*

Cholera case No.	Age.	Interval between attack and admission.	Pulse on admission.	Urination on admission.	Manner of treatment.	Month of pregnancy	Recovered in—	Died of
		Hours.					*Days.*	
6159	21	4	Present	Absent	Dilatation and extraction.	7	7	
6170	23	7	do	do	do	5	7	
6189	30	36	do	do	do	7	10	
6197	26	8	Absent	do	Dilatation	4	6	
6332	30	8	Present	do	do	5	9	
6345	30	12	Absent	do	Dilatation and extraction.	7		Pneumonia 29 days after.
6381	18	18	Present	Present	do	[a]8	5	
6395	20	4	Absent	Absent	do	7		Uræmia 12 days later.
6439	24	?	Present	do	Dilatation	3	10	
6413	21	5	Absent	do	Dilatation and extraction.	8	9	
6443	25	12	Present	do	Dilatation	5	12	

[a] Fœtus was alive when born.

The above 11 cases of pregnant cholera women, in which ten of the fœtuses were dead, were aided either by dilatation of the cervix uteri alone or by dilatation and extraction of the fœtus. All were in a very serious condition. In 7 of these, dilatation and extraction were performed. The average age was 24 years;

the average period of pregnancy was 5.7 months; the average interval from the beginning of the attack until admission to the hospital was only 10.5 hours, a substantiating fact that the fœtuses are killed early in the first stage of the disease. Two cases in which dilatation and extraction were performed died, one of pneumonia twenty-seven days later and the other of uræmia nine days later. The latter patient had passed a normal amount of urine for four days and was on full diet for two days, when she developed such a severe case of uræmia that all our efforts failed to save her. All the other cases made uneventful recoveries.

In the cases in which dilatation and extraction were performed, the whole operation did not take more than fifteen minutes for any one case. No anæsthesia was necessary, as the patients were in the partially comatose state seen so frequently in cholera. Also, as the tissues were very lax, the cervix uteri was very easily dilated, and the shock to the mother was practically nil. The fœtuses with one exception were dead when removed and showed no signs of maceration. The amniotic fluid was almost normal in amount, and the after-delivery bleeding was always very scant, except in the case of the birth of the live fœtus, in which it was slight. The procedure in the last-mentioned case was resorted to because abortion was in progress, and if not speedily terminated, the patient would have died.

The mortality of these 11 cases was 18 per cent, which is about the same obtained in the nonpregnant cases in this hospital (19 per cent), thus giving the pregnant patient the same chance for recovery as the nonpregnant. This procedure prevented the mortality of 45 per cent, which was obtained in the pregnant cases not treated in this manner. The average period of recovery was 8.7 days, which, although not as low as the average 7.3 days that was obtained in those cases that did not abort, is lower than the average in those cases that did abort without this procedure, which was 9.6 days.

Nichols(12) prefers not to interfere with the pregnant condition of cholera patients on the grounds that they are not strong enough to withstand the shock. This is very true at times if the interference is not resorted to until the patient is in an extremely serious condition, as I have seen on three occasions. Basil(1) states that he cannot follow the advice of some of the continental authorities who advocate emptying the uterus in all cases. This seems to be unwise, as there is a class of patients who will recover without this procedure. During

this same period there were also admitted 6 cases whose condition was such that the pregnancy showed no signs of being interrupted, and they all were discharged with their fœtuses intact. French(4) agrees with Basil(1) and states that convalescence is delayed when the pregnancy is interrupted, but in the strict sense of the word none of these cases was interrupted, but the mother was simply helped to remove the dead fœtus, which, if she were left alone, would have in all probability taxed her strength to the limit to have delivered it later, and in the meantime she would have absorbed its toxins to her own detriment.

From the facts narrated above, it is evident that the speedy termination of attempted abortion, or the removal of the dead fœtus, is not only justified but indicated. Even if the number of cases experimented upon may seem to be small, I think it is sufficiently large when considering the results obtained. The almost immediate urination following and the general change in the condition for the better after the delivery were at times nothing short of marvelous.

This method seems to be original, as I am unable to find any mention of it in the literature available, with the possible exception of Davis,(3) who advocates the speedy termination of the labor if it should start, but says nothing about removing the dead fœtus when abortion is not threatened.

CONCLUSIONS

1. Pregnant cholera cases have a higher mortality than nonpregnant cases, if left to their own resources.

2. The later the pregnancy the graver is the prognosis for the mother.

3. There is some factor other than mechanical which kills the fœtus very early in the disease in the majority of the cases.

4. Abortions occur in most of the cases, and the older the fœtus the greater is the tendency to abort.

5 Most of the pregnancies come to a fatal termination.

6. Abortion is nature's therapeutic measure in aiding the mother in her fight for life.

7. The essential factor in the treatment of pregnant cholera cases is to remove the dead fœtus as soon as possible and in the manner best suited to the mother's condition, because it shortens the period of convalescence, preserves the strength of the mother, and reduces the mortality to about that of the nonpregnant cases.

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ECHINOSTOMA ILOCANUM (GARRISON): A REPORT OF FIVE CASES AND A CONTRIBUTION TO THE ANATOMY OF THE FLUKE [1]

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ONE PLATE AND ONE TEXT FIGURE

In October, 1916, an ovum was found in the clinical laboratory of the Philippine General Hospital, Manila, measuring 90 microns in length and 50 microns in width and bearing the same morphological characteristics as described by Garrison in 1907.

> It is oval in shape with one end more sharply rounded; the shell is light brown in color, smooth and rather delicate, with an operculum at the sharper end; the contents are rather refractile, colorless, and composed of a mass of yolk-cells, among which the germ cell could in some cases be recognized.

Large numbers of ova of this description were collected from the first two cases, and they were placed in distilled water and in saline solution and were preserved in alcohol after fixation in acetic sublimate.

After a careful study of the material on hand a tentative diagnosis of *Echinostoma ilocanum* was made, which was later confirmed by Professor Crowell and one of us (Wharton) in a fresh specimen.

Through the courtesy of Dr. E. Domingo, from the department of medicine, it was possible for us to secure the worms after a dose of male fern. Eight worms were obtained from the first case, the specimen of stool from the second case failing to show any. The treatment consisted of 24 capsules of oleoresin of male fern given in doses of 4 capsules every ten minutes. During the previous day the patient was kept on liquid diet without milk as a preparatory measure for the next day's treatment.

The detection of the worm in the stool is fraught with no little difficulty on account of its small size, its flat body, and its appearance, which makes it hardly distinguishable from the small parti-

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cles of fæcal matter. This is especially true if the worm is more or less decomposed, when it is rather flabby and pale; it is relatively easily recognizable when fresh or when suspended in formalin solution as passed with the stool. It then shows a grayish color, usually with injected coils along the median line, plainly visible through the cuticle.

To obtain better specimens than the first eight, on which no spines on the cuticle were seen, as described by Odhner, except one, which showed them around the oval sucker, male fern was again given the same two patients from whom we secured the first batch of worms. This time the stools were collected in vessels containing 10 per cent formalin solution, and eight apparently well preserved flukes were recovered from both. They were immediately examined under the microscope; only one showed cuticular spines. In December, 1916, ova of identical description were found again in the course of routine examination of fæces from three charity patients, who were admitted in the free wards for diseases alien to the presence of the worm in the intestines, such as adenocystoma of ovary. Of these only one was treated with male fern, but the specimen was lost, owing to a mistake on the part of the personnel in charge, and further treatment could not be undertaken because of the patient's poor condition.

The symptoms presented by these five cases at the time their stools were examined were anæmia, fairly marked in one of them, with occasional headache and dizziness. The blood picture showed no other changes, outside of a more or less marked decrease of the hæmoglobin percentage, moderate diminution in number of red blood corpuscles, and eosinophilia in some cases. In two cases *Ascaris* and *Trichuris* ova were found as well; in one case, *Ascaris*, *Trichuris*, and hookworm, and in two the fluke was present alone. All the cases are natives of Zambales, Luzon, the towns whence they came being San Felipe, San Antonio, San Narciso, and Cabañgan.

No definite pathological changes have been described as yet as being brought about by the invasion of the intestines by this worm. The fact that it apparently thrives on the host's blood, as shown by the constant presence in the hosts of varying degrees of anæmia associated with reflex symptoms and slight variation in the blood picture peculiar to blood-sucking intestinal parasites, and the perfusion of the digestive tract of the fluke, which has been observed on examination of the fresh specimen, are very significant.

That the fluke is a common parasite in the intestines of natives

of Zambales, we believe, is not to be questioned. The fact that all our cases came from that province tempts us to assume that the parasite may be just as prevalent there as in Ilocos Sur, which is the locality established by Garrison on the basis of the fact that all his cases were obtained from there. In this connection further work on geographical distribution of the fluke may show, in the face of the conflicting findings as to locality, wider fields of distribution than is even now suspected.

REPORT OF CASES

Case 1.—I. F., female, 20 years old, born and living in San Felipe, Zambales, entered the School of Nursing July, 1916. She is moderately well nourished, but very pale. She complains of constipation, headache, and occasional dizziness. Blood examination shows 65 per cent hæmoglobin, 4,300,000 erythrocytes, 8,600 leucocytes, 78 polymorphonuclear neutrophiles, 20 lymphocytes, 8 polymorphonuclear eosinophiles, 4 large mononuclears, In October, 1916, in the routine examination of the stool, a moderate number of ova of *Echinostoma ilocanum* was encountered. After giving a dose of oleoresin of male fern, 8 worms were obtained, in apparently poor condition. On January 21, 1917, the treatment was repeated and the stool was collected in a dish containing 10 per cent formalin. Five worms in apparently good condition were passed. Of these only one exhibited cuticular spines. The specimen was fixed in formalin solution.

Case 2.—T. M., female, 21 years old, native of, and residing in, San Antonio, Zambales, entered the Philippine General Hospital as a probationary student in the School of Nursing on July, 1916. She is well nourished and well developed, but somewhat pale. She complains of occasional headache, sometimes stomach ache, regularly slight constipation, and irregular menses. Blood examination showed 85 per cent hæmoglobin, 5,400,000 red blood cells, 11,600 leucocytes, 78 polymorphonuclear neutrophiles, 15 lymphocytes, 6 large mononuclears, and 1 eosinophile. In November, 1916, the examination of the stool showed rare ova of *Echinostoma,* as also a few eggs of *Ascaris* and *Trichuris.* She was given a treatment of male fern. After a careful examination of the stool no worms were found. On January 21, 1917, another dose of male fern was administered, and the stool was directly received in a solution of formalin. Three worms were recovered.

Case 3.—(Case 47963) P. C., 38 years old, female, married, born and living in Cabangan, Zambales, admitted to the Philippine General Hospital for enlarged abdomen. She is fairly well

developed, but much emaciated, with œdema of the limbs. Laparatomy showed a large tumor in the region of the ovary. On January 18 the stool was sent in for routine examination. A few ova of *Echinostoma ilocanum* were found. Male fern treatment was given, but for reasons already set forth, the specimen of stool collected was not examined for the worm.

Case 4.—(Case 6920) C. V., male, 10 years old, born and residing in San Narciso, Zambales, admitted to the Philippine General Hospital complaining of yaws. He is fairly well developed and well nourished, but somewhat pale. Blood picture shows 75 per cent hæmoglobin; otherwise it is normal. On December 7 examination of the stool showed a few eggs of *Echinostoma ilocanum*. No treatment for worm was given, because the patient left the hospital the day after the examination. A large number of eggs of *Ascaris* and occasional eggs of hookworm and *Trichuris* were found.

Case 5.—We could not secure authority to publish the clinical record of this case.

THE ANATOMY

Historical.—Echinostoma ilocanum was first discovered by Philip E. Garrison in Ilocano prisoners in Bilibid Prison, Manila, in 1908. He found the eggs in 5 cases and on treating his last case with male fern recovered 21 worms from the stools. He described the worms as a new genus and species, giving them the name *Fasciolетta ilocana*. Later he sent 4 specimens to Doctor Odhner, at Upsala, and in a paper in the Zoologischer Anzeiger, in 1911, Doctor Odhner pointed out several new features of the anatomy and showed that the worms belong to the old genus *Echinostoma* whose type species is *Echinostoma echinatum* (Zeider 1903) found in domestic ducks and geese. Since that time there are no reports of the finding of this worm, although Willets reports finding similar eggs in one case in Cagayan.

Another species, *Echinostoma malayanum*, was reported in 1912 by Leiper from natives of Singapore and the Federated Malay States.

Dimensions.—In fifteen specimens measured Garrison found a maximum length of 6 millimeters and a maximum breadth of 1 millimeter and a minimum of 4 millimeters by 0.75 millimeter. We have found a somewhat greater range in the size of our specimens. The dimensions of eight worms, the first three collected on November 2 and the others on January 22, were as follows:

TABLE I.—*Dimensions of 8 worms.*

	Length. mm.	Maximum breadth. mm.
No. 1	4.03	0.98
No. 2	4.53	1.15
No. 3	4.80	1.34
No. 4	4.36	1.39
No. 5	5.16	1.33
No. 6	5.82	1.53
No. 7	6.46	1.46
No. 8	7.82	1.60

The greatest width of the body is at the level of the ventral sucker, from which point the body tapers gradually to the rounded posterior end. In front of the sucker is the head region, which is generally bent dorsally at the level of the genital pores; there is often a distinct constriction at this point. The shape and length of the head region vary considerably in different specimens due to the difference in the state of contraction of the worms. At the ventral sucker the body is almost round in cross section, but it becomes flattened rapidly toward each extremity.

Color.—There is very little pigment in the body wall. The fresh specimens were a transparent gray, and the testes, ovary, and uterine coils could be seen distinctly through the walls.

Cuticular spines.—Garrison says "the cuticle is smooth and without spines." Odhner describes a well-developed armor of scalelike spines which he says covers the anterior part of the body and is perceptibly developed on the margins of the body as far back as the border of the posterior testicle. We find in our specimens a very remarkable variation in regard to this feature of the body. In a few specimens the anterior part of the body was fairly well covered with very small spines, but in a majority of cases these spines are entirely lacking or are found only in irregular patches along the margins of the body. They are very unstable, and the least handling causes them to be lost. The cuticle of two of our best preserved specimens is smooth and shows no indications, even under a magnification of 400 diameters, of ever having borne spines at any time. It may be that the spines are developed and lost very early in the life of some worms, or some may fail entirely to develop spines at any time. When present they are so small that it is impossible to see how they could be of any use to the worms.

Oral spines.—Another variable feature of the anatomy is the presence or absence of the wreath of oral spines around the

mouth and anterior sucker, which is the most important characteristic of the genus *Echinostoma*. Garrison failed to note these spines in any of the specimens which he studied, and Odhner says of the four specimens sent him by Garrison that "the arrangement of the spines can only be followed in one specimen." From our 16 specimens we obtained only 3 in which any traces of these spines could be seen, and in only one specimen were they in a condition such that their arrangement could be studied. It seems that these spines, although much larger, are even more unstable than those of the cuticle (Plate I, fig. 1).

In the specimen described by Odhner 49 spines were present, which were arranged in the form of a wreath around the oral

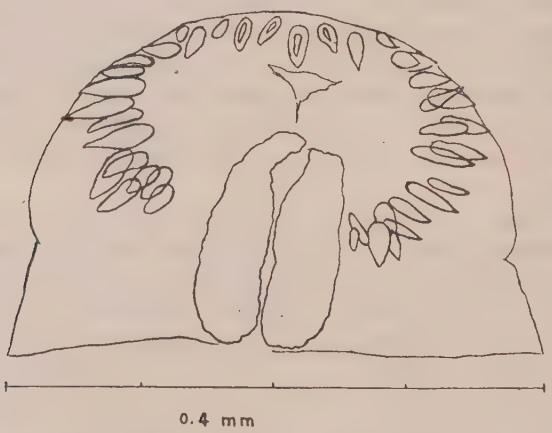

FIG. 1. Outline drawing of anterior end of *Echinostoma ilocanum* (Garrison), showing the arrangement of the oral wreath of spines.

sucker, broken on the ventral side. There were two corner groups next to the ventral opening of 5 and 6 spines, respectively, then 2 single spines on each side, followed by 10 spines arranged in pairs, and finally 14 single spines forming the dorsal part of the wreath. The arrangement of the wreath in our specimen may be seen in fig. 1. As will be seen, there are only 39 spines present, but their arrangement is very much the same as described by Odhner. There are the two corner groups, the single spines, then the paired spines, and the row of dorsal spines. From a study of their arrangement it can be seen that several spines on our specimen have been lost.

The size of the spines in different parts of the wreath varies considerably. The largest ones in the corner groups are 50 mi-

crons long by 10 microns wide at the base. Then there is another type of a shorter, thicker form, 44 by 18 microns, and still smaller forms found in the corner groups and in the dorsal row, which average about 29 by 15 microns.

In those specimens where this wreath is not present no indication of such a structure can be seen, so it is not surprising that it was overlooked by Garrison. A drawing of a specimen without the spines is shown in Plate I, fig. 2.

Ventral sucker.—The ventral sucker is the most prominent feature of the surface of the body, the average size of this organ in 6 specimens being 530 microns in diameter. The center of the sucker lies at about the middle of the anterior third of the body.

Alimentary tract.—The oral sucker measures 120 to 200 microns in diameter and is slightly subterminal in position. It opens into a short prepharynx. The pharynx is globular in contracted specimens and has an average diameter of about 160 microns. In those specimens in which the head region is extended, the pharynx is considerably longer and decreases slightly in transverse diameter. The œsophagus is short (50 to 120 microns) ; it bifurcates just in front of the genital pores. The two unbranched intestinal cæca pass transversely outward to near the margins and then run posteriorly almost to the end of the body in the marginal region. In the posterior part of the body they are almost surrounded by the vitelline glands.

Excretory system.—The excretory pore is in the middle of the posterior border and opens into a large median excretory tube which runs forward to the posterior border of the posterior testicle, where it divides into two branches which run forward between the intestinal cæca and the reproductive organs. Thus they divide the body into three distinct regions, two lateral ones which contain the intestinal cæca and the vitelline glands and a median region containing the genital organs. In the region of the ventral sucker the tubes increase in diameter and appear to unite dorsad to the sucker.

Male genital organs.—The testes lie in the posterior half of the middle region of the body, one behind the other. In some specimens they are oval and appear as solid masses, but generally they are elongated and are distinctly divided into anterior and posterior lobes by a transverse constriction. From each testicle a vas deferens runs forward near the margin of the genital region. They unite dorsad to the posterior sucker and enter the posterior end of the cirrus pouch. The opening of the cirrus

pouch lies just back of the bifurcation of the intestine and in front of the ventral sucker, and the pouch extends back dorsad to the sucker, being about twice as long as it is wide. In its posterior part is a seminal vesicle, into which the vasa deferentia open. It is capable of considerable distention, so that in some cases it nearly fills the cirrus pouch, while in others it seems to be very small. It opens directly into a muscular cirrus, which is long and spirally coiled and often is extended through the genital pore.

Female genital organs.—The ovary is globular and is situated a little to the right of the median line at about the middle of the length of the body. Its oviduct passes into a rounded "shell gland," which lies between the ovary and the anterior testicle in the middle line of the body. The vitelline glands commence about midway between the posterior border of the ventral sucker and the anterior border of the ovary. They extend to the posterior end of the body. In the anterior part they lie entirely in the lateral margin, but as they pass back, they spread out dorsally and ventrally and almost completely surround the body posterior to the testes. The transverse vitelline ducts turn inward just anterior to the testes and enter the shell gland posterolaterally. A receptaculum seminis is not present. The uterus leaves the shell gland on the left side and turns anteriorly to in front of the ovary, where the coils begin. They fill all of the space in the middle region of the body between the ovary and the ventral sucker. The anterior part of the uterus forms a well-developed vagina, which passes forward dorsad to the ventral sucker and opens at the female genital pore, which lies just to the left of the male pore.

Ova.—The ova have been well described by Garrison in his paper. They have a thin shell with an operculum at the smaller end and are not segmented when passed in the fæces. The variation in length and breadth is considerable, but from a measurement of 25 fresh eggs, we get nearly the same average size as Garrison. His measurements are:

Maximum length, 114.7; breadth, 81.9 microns.
Minimum length, 88.8; breadth, 53.5 microns.
Average length, 99.58; breadth, 56.04 microns.

Our measurements are:

Maximum length, 111; breadth, 74.4 microns.
Minimum length, 88.8; breadth, 53.6 microns.
Average length, 101.21; breadth, 56.4 microns.

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ILLUSTRATIONS

[Drawings by Castro with camera lucida outlines.]

PLATE I

FIG. 1. *Echinostoma ilocanum* (Garrison), with oral spines. In this specimen the vitellaria are not well shown.

2. *Echinostoma ilocanum* (Garrison), without oral spines.

TEXT FIGURE

FIG. 1. Outline drawing of anterior end of *Echinostoma ilocanum* (Garrison), showing the arrangement of the oral wreath of spines.

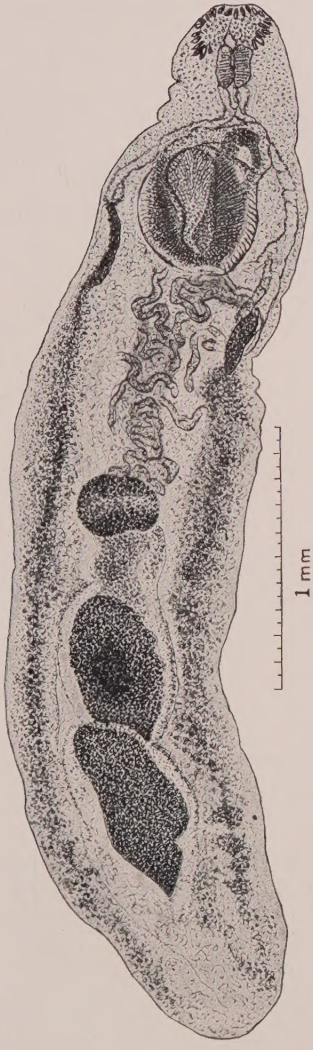

Fig. 1.

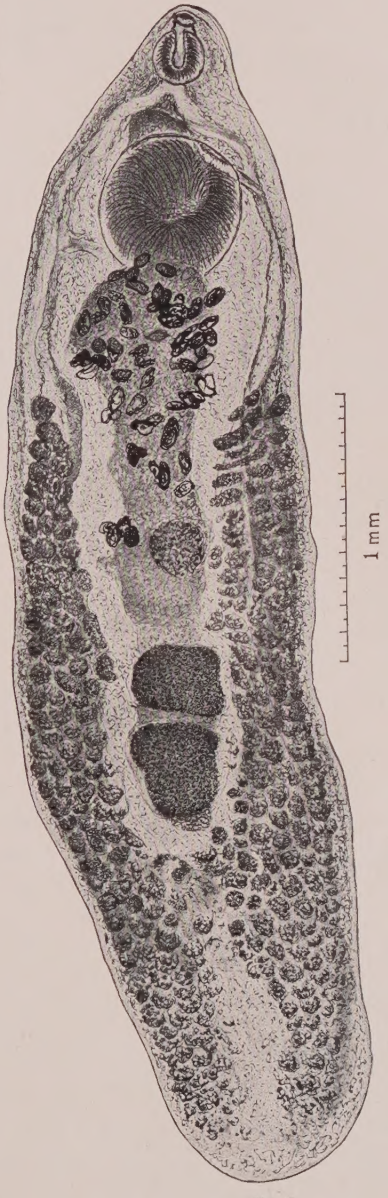

Fig. 2.

PLATE I. ECHINOSTOMA ILOCANUM (GARRISON).